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Negligible risks to corals from antifouling booster biocides and triazine herbicides in coastal waters of the Chagos Archipelago

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The Chagos Archipelago is the most isolated and biologically diverse group of atolls in the central Indian Ocean. It contains 5 islanded atolls, including the world's largest atoll, the Great Chagos Bank. Following the abandonment of the copra plantations, in 1973, most islands are uninhabited and have rarely been visited. The only exception is Diego Garcia, part of which is a strategic military base with appropriate infrastructure to support long range aircraft and ships which visit and reside for extended periods in the lagoon which provides anchorage.

In 1996, a research programme was undertaken in Chagos (Sheppard and Seaward, 1999) to assess the atolls biodiversity, biogeographic role with respect to fisheries, and the degree of their pristine condition. Analyses of sediment samples from throughout the Archipelago revealed that hydrocarbons present were predominantly of biogenic origin (Readman et al., 1999). However, these studies also revealed some petrogenic and pyrogenic polycyclic aromatic hydrocarbons (PAHs) at sub to low ng g^{-1} dry sediment. Organochlorine analyses revealed that only some polychlorinated biphenyl (PCB) congeners and the pesticide lindane were above limits of detection. It was concluded that atmospheric transport (from industrial parts of the world) was the major route for the introduction of organochlorines to the region. A parallel study (Everaarts et al., 1999), based upon analysis of anthropogenic organic contaminants and toxic metals in sediments and biota, confirmed that, at least in 1996, Chagos belonged to the category of least impacted coastal areas.

During recent years, antifouling products used in boat paints have tended to incorporate organic "booster" biocides to enhance performance and to move away from tributyltin compounds (which were restricted in most countries due to their demonstrated toxicity to non-target organisms at very low concentrations). The booster biocide products are primarily herbicidal and contain active ingredients including: Irgarol®1051 (2-methylthio-4-*tert*-butylamino-6-cyclopropylamino-*s*-triazine), Sea-Nine 211®/kathon 5287 (4,5-dichloro-2-*n*-octyl-4-isothiazolin-3-one), chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile), dichlofluanid (1,1-dichloro-*N*-[(dimethylamino)sulfonyl]-1-fluoro-*N*-phenylmethanesulfenamide), diuron (DCMU, [3-(3,4-dichlorophenyl)-1,1-dimethylurea]), TCMS pyridine, TCMTB, zinc pyrithione and zineb.

Irgarol®1051 has recently been shown to be a potent inhibitor of coral photosynthesis in both isolated endosymbiotic coral zooxanthellae (i.e. isolated algal symbionts *in vitro*) and in the intact coral symbionts (i.e. *in vivo*) for a number of Atlantic and Pacific coral species (Owen et al., 2002, 2003; Jones and Kerswell, 2003; reviewed by Jones (2005)). In *in vitro* experiments, Owen et al. (2002) report no $C^{14}(H^{14}CO_3^-)$ incorporation in zooxanthellae isolated from the common branching coral *Madracis mirabilis* after a 6 h exposure to 63 ng l^{-1} Irgarol 1051®. *In vivo*, the

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photochemical efficiency of the symbiotic algae in *Seriatopora hystrix* has been shown to be significantly reduced following several hours exposure to Irgarol®1051 at concentrations as low as 50 ng l^{-1} (Jones and Kerswell, 2003). The toxicity of Irgarol®1051 demonstrated in these studies is generally higher that those reported for other PSII inhibiting triazine (e.g. atrazine and simazine) and non-triazine herbicides.

The aim of the present study was to investigate the presence of selected booster biocides (Irgarol®1051, chlorothalonil, dichlorofluanid and Sea-Nine 211®) associated with vessels visiting/anchoring within the Diego Garcia harbours to assess potential risks to corals. In addition, our analytical protocol also screened for three other triazine herbicides used primarily for weed control (atrazine, simazine and ametryn). Finally, full scan GC–MS chromatograms were scrutinized for other compounds co-extracted during the C18 solid phase extraction (SPE).

Sampling was conducted during February and March 2006 and was focused on Diego Garcia and, more specifically, on the harbour areas. Replicate sea water samples (typically $n = 3$) were taken at 14 coastal locations, including an oceanic reference site located 5 miles north of the island (Table 1; Fig. 1). A tidal cycle sampling regime was performed at Moody Brook (station 12, a small boat harbour) to investigate fluxes in the lagoon. Sea water samples (2 l) were collected at 30 cm below the surface in pre-cleaned (acid soaked and high purity solvent rinsed) amber bottles. To avoid contamination, bottles were filled from the front of small inflatable boats whilst gradually moving into the prevailing wind. Samples were processed within a few hours of sampling. Samples were spiked with terbutryn (Riedel-de Haën) as an internal standard to assess recovery (250 ng terbutryn added to yield a concentration of 125 ng l^{-1}).

The antifouling booster biocides were quantitatively extracted from the water samples by solid phase extraction (SPE) using Isolute® Triazine SPE cartridges (6 ml–500 mg sorbent mass, Argonaut Technologies). Each cartridge was conditioned with 10 ml of HPLC grade methanol (Rathburn Chemicals Ltd.), followed by 10 ml of deionised water at a flow rate of 10 ml min^{-1}. Sea water samples were then passed through the conditioned SPE cartridges at a constant flow rate of 15 ml min^{-1}. At the end of the extraction, 10 ml of deionised water was used to wash the cartridges, after which they were vacuum air dried for 30 min. Cartridges were then frozen (at <-20 °C) until subsequent analyses were performed at the laboratory.

Table 1
Sample locations and environmental conditions

Station no.	Location	GPS position	Date	Tide	Conditions
1[a]	Central small boat harbour	S 07 17.478 E 72 23.490	20/2/2006	HW + 3h	Calm. light rain
2[a]	Entrance small boat harbour	S 07 17.360 E 72 23.448	21/2/2006	HW + 1h	Swell. heavy rain
3	T-site pier	S 07 17.362 E 72 23.816	22/2/2006	HW + 15m	Slight swell. Rain
4	1/2 way T-site pier and anchorage	S 07 16.954 E 72 24.101	22/2/2006	HW + 15m	Slight swell. Rain
5	Anchorage east of T-site	S 07 16.519 E 72 24.477	22/2/2006	HW + 15m	Slight swell. Rain
6[a]	N-W of Small Boat Harbour	S 07 16.971 E 72 23.345	23/2/2006	HW − 2h20	Calm. clear
7	Turtle Cove	S 07 25.551 E 72 26.137	23/2/2006	HW − 7h	Clear, choppy
8	North of Turtle Cove	S 07 24.150 E 72 26.373	23/2/2006	HW − 6h50m	Clear, choppy
9	Middle of Central Basin	S 07 22.223 E 72 26.657	23/2/2006	HW − 6h40m	Clear, choppy
10	East of Point Marianne	S 07 19.168 E 72 27.194	23/2/2006	HW − 5h10m	Clear, choppy
11	South East of POL pier	S 07 18.157 E 72 26.378	23/2/2006	HW − 4h55m	Clear, choppy
12[b,c]	Moody Brook, Small Boat Harbour	S 07 17.475 E 72 23.432	22/3/2006 25/2/2006	HW − 2h HW − 3h, HW, HW + 3h, LW	Clear, calm Clear, calm
13[d]	Approx 5 miles north of Diego Garcia atoll entrance		22/3/2006	HW + 4h	Clear, moderate swell
14	Beach in front of BOQ 3	S 07 15.880 E 72 22.685	22/3/2006	HW − 1h30m	Clear, calm

[a] $n = 3$.
[b] Tidal cycle sampling.
[c] $n = 2$.
[d] $n = 4$.

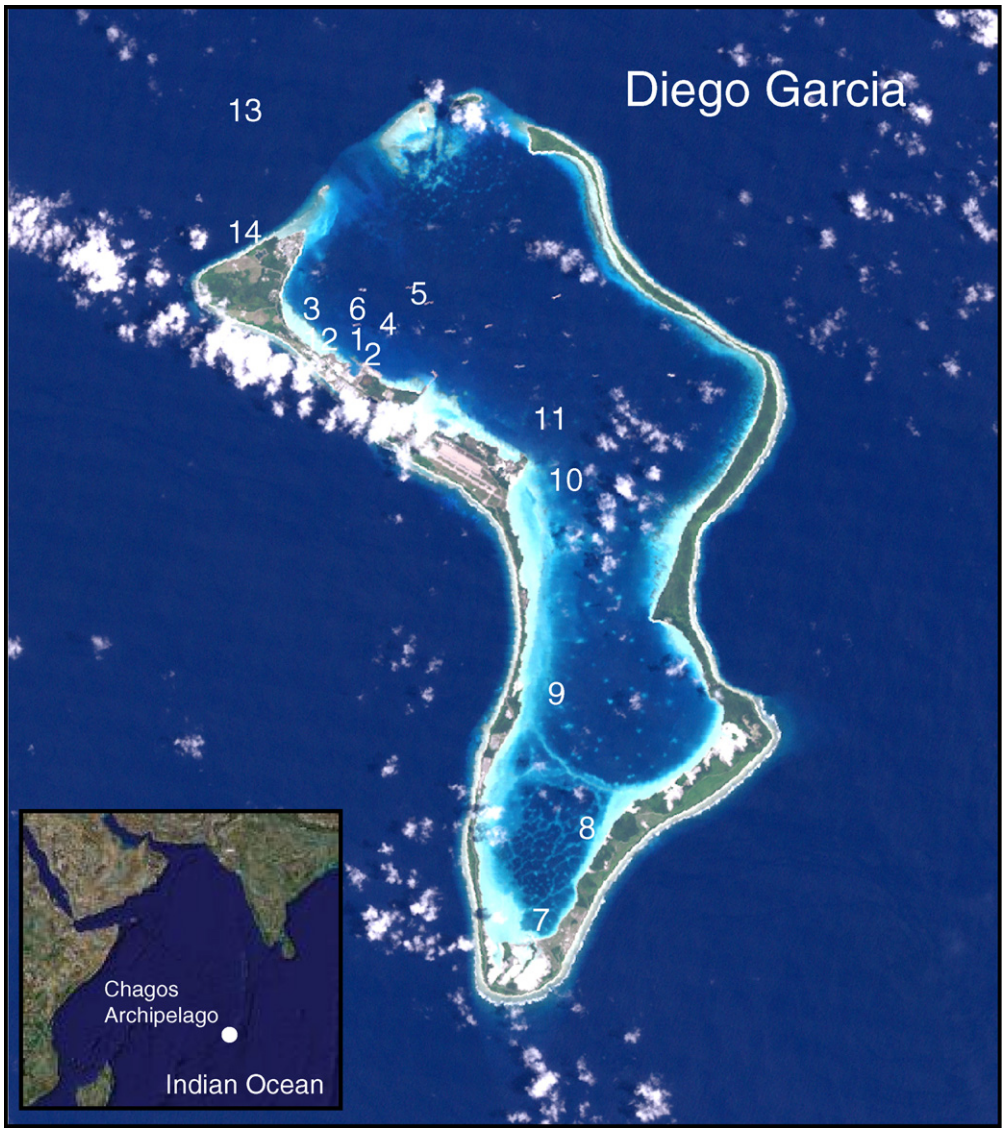

Fig. 1. Satellite image of Diego Garcia showing the approximate locations of the sampling sites. (Precise GPS positions are provided in Table 1.)

Using a VacMaster® sample processing station, cartridges were eluted with 6 ml of HPLC grade dichloromethane (Rathburn Chemicals Ltd.) and the eluents were dried using in-line Isolute® sodium sulphate cartridges (2.5 g Na_2SO_4/cartridge, Argonaut Technologies). Atrazine-D5 (Qmx Laboratories) (20 µl of 10 ng μl^{-1} solution) was then added to each eluent as a second internal standard to evaluate absolute recovery. Samples were blown down to approximately 250 µl under ultra high purity nitrogen and were then transferred into GC micro-vials. Method blanks were simultaneously run with each batch of samples.

Analyses were undertaken using an Agilent 6890 Gas Chromatograph with a 5973 Mass Selective Detector equipped with an inert source, an auto-sampler, and fitted with a HP-MS 5 capillary column (5% diphenyl/95% dimethylsiloxane; 30 m × 0.25 mm i.d., 0.25 µm film thickness) in rapid scan simultaneous selected ion-monitoring mode (SIM) and full scan acquisition. The GC inlet was operated in splitless mode with a 2.0 µl injection volume and an injector temperature of 280 °C. Helium carrier gas (maintained at 1 ml/min) was used. The oven was ramped at 20 °C min^{-1} from an initial starting temperature of 80 °C (held for 1 min) to 200 °C. The temperature was then increased at 8 °C min^{-1} to 300 °C. Run times were 19.5 min. Target and qualifier ions placed within the SIM descriptor were: for Irgarol®1051 *m/z 253*, 182 and 238; for Sea-Nine 211® *m/z 246*, 169 and 182; for chlorothalonil *m/z 266*, 264 and 268; for dichlofluanid *m/z 224*, 123 and 167; for atrazine *m/z 215*, 202 and 200; for simazine *m/z 201*, 186 and 173; for ametryn *227*, 212 and 170; for terbutryn *m/z 241*, 185 and 226; and for atrazine-D5 *220*, 207 and 205.

Standards were run prior to sample analyses to calibrate the instrument. Calibration standards were run for QA/QC verification during each sequence, as well as reagent, methodological and field blanks to investigate potential contamination. The theoretical detection limits of the protocol

were: Irgarol 1051®, 0.4 ng l^{-1}; Sea-Nine 211®, 0.9 ng l^{-1}; chlorothalonil, 0.1 ng l^{-1}; dichlofluanid, 0.1 ng l^{-1}; atrazine, 0.4 ng l^{-1}; and simazine, 0.9 ng l^{-1}. 1 ng l^{-1} was selected as a conservative limit of detection for all compounds. The full scan GC/MS data was used for validation purposes. Irgarol®1051, Sea-Nine 211®, chlorothalonil, dichlofluanid, atrazine, simazine and ametryn were not present in any of the cartridge blanks or offshore reference sea water samples (5 miles north of Diego Garcia). In addition, two samples taken from 2 locations (stations 12 and 14), were not spiked with the internal standard and confirmed the absence of terbutryn in the samples.

Analytical recoveries of the compounds were assessed through extraction and analyses of offshore sea water spiked with authentic standard compounds [Irgarol®1051 (Ciba Geigy Ltd.); chlorothalonil, dichlofluanid, atrazine, simazine, ametryn (Riedel-de Haën); Sea-Nine 211® (Rohm and Haas)]. Mean relative recoveries for the concentration range from 25 ng l^{-1} to 150 ng l^{-1} were as follows: Irgarol 1051® 89% ± 10%, Sea-Nine 211® 79% ± 12%, chlorothalonil 95% ± 8%, dichlofluanid 68% ± 5%, atrazine 74% ± 7%, simazine 52% ± 10%, and ametryn 62% ± 4%. Data for the environmental samples were corrected for recoveries.

Concentrations of the antifouling booster biocides and herbicides analysed were extremely low. Indeed, of these substances screened for, only Irgarol®1051 was detected. Even for this compound, it was only encountered in 2 of the 31 samples analysed. One replicate from Station 1 contained 2 ng l^{-1} Irgarol, and one replicate from station 2 contained 8 ng l^{-1} Irgarol. Other than these two, all other 29 samples from 14 sites (including other replicates from Stations 1 and 2 and the tidal cycle samples) contained

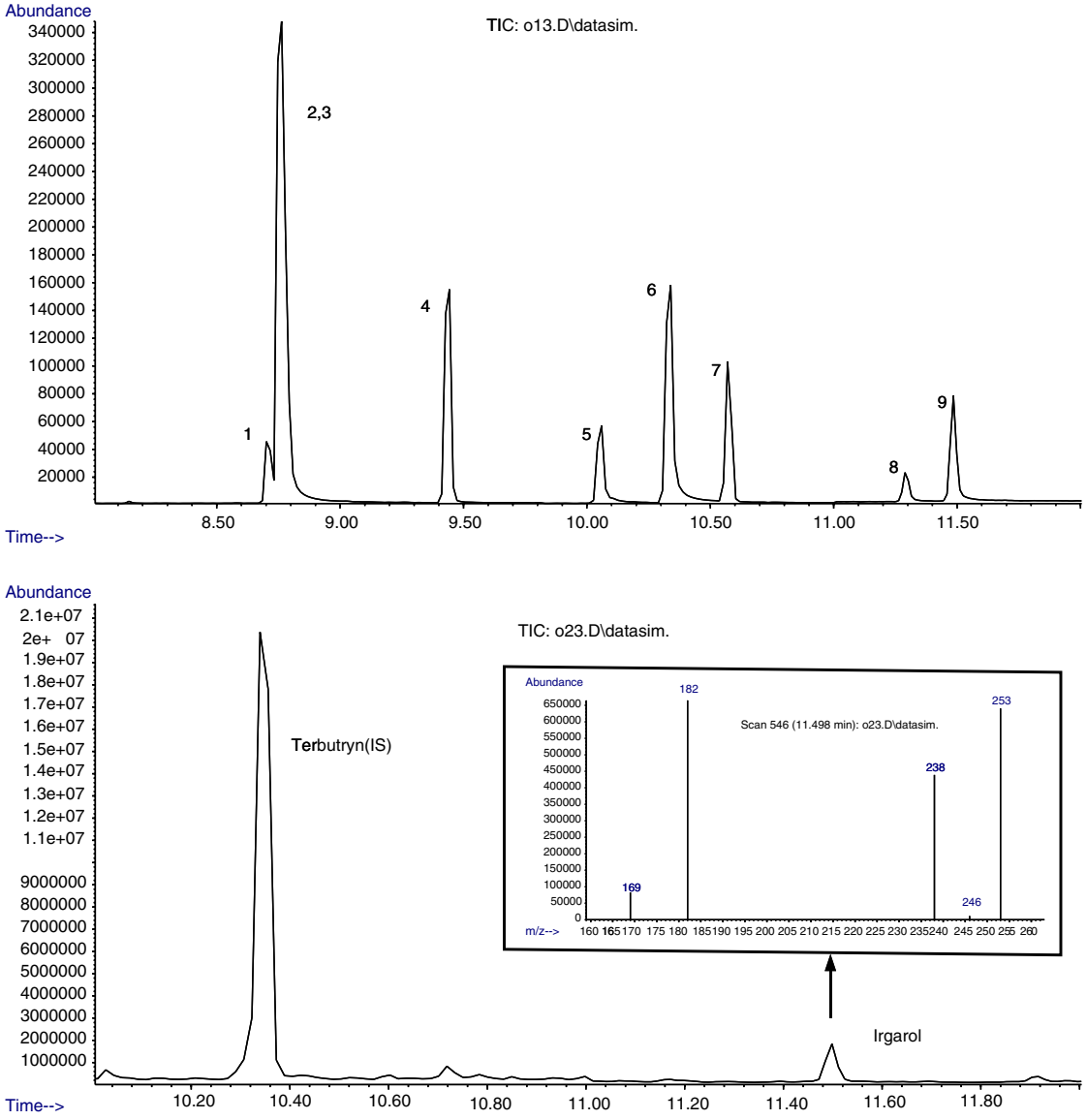

Fig. 2. Total ion current GC–MS chromatograms of standard compounds (1 = simazine; 2 and 3 = atrazine and atrazine D_5; 4 = chlorothalonil; 5 = ametryn; 6 = terbutryn; 7 = dichlofluanid; 8 = Sea-Nine 211®; and 9 = Irgarol®1051) and the extract from station 2 showing the presence of Irgarol®1051 (8 ng l^{-1}). An inset mass spectrum of the peak confirms the correct ion ratios.

<1 ng l^{-1} of Irgarol 1051®, chlorothalonil, dichlofluanid, simazine, ametryn, atrazine and Sea-Nine 211. Examples of a booster biocide standard chromatogram together with the verification of Irgarol®1051 in the sample from station 2 are shown in Fig. 2. The lack of matrix interferences afforded excellent sensitivity and detection limits. SPE cartridge blanks, although contaminated (e.g. with plasticizers), did not interfere with quantification of the selected compounds. In addition, the cartridge blanks were consistent, affording screening for additional environmental materials in the sample extracts.

The results indicate negligible contamination of the Chagos coastal waters from any of the biocides analysed. For comparison, levels of booster biocides from through-

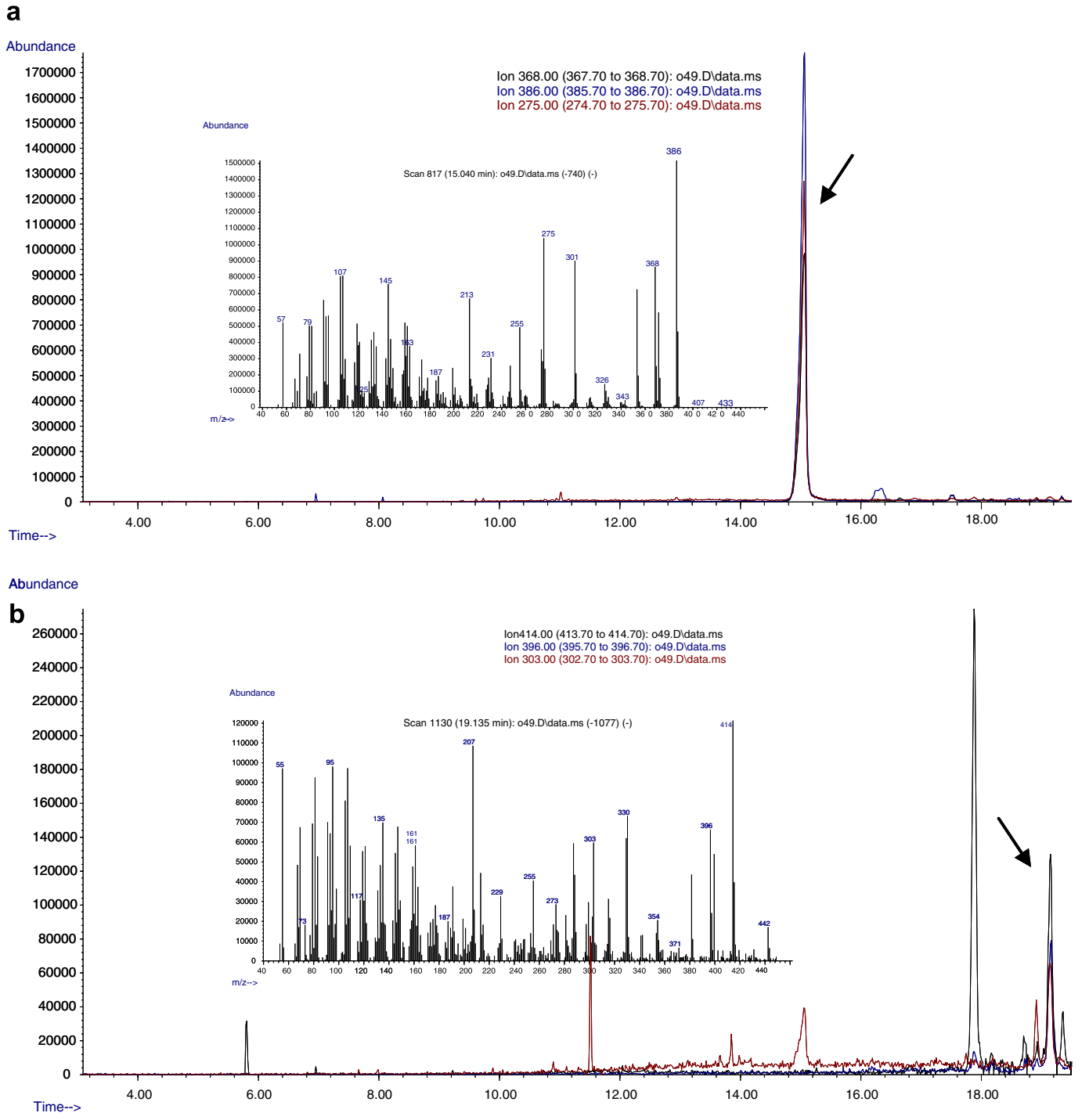

Fig. 3. Selected ion monitoring GC–MS chromatograms of sterols detected in the sample extracts, with inset mass spectra to confirm identities. (a) is cholesterol (cholest-5-en-3β-ol) and (b) is β-sitosterol (24α-ethylcholest-5-en-3β-ol). The m/z 414 ion peak eluting prior to β-sitosterol (b) was not identified.

out the world have been reviewed (Konstantinou and Albanis, 2004). The vast majority of the available data is for Irgarol®1051, and primarily for temperate locations. Some studies have, however, been undertaken in tropical and sub-tropical areas. Concentrations of Irgarol®1051 up to 590 ng l^{-1} have been reported for coastal waters in Bermuda (Connelly et al., 2001; Owen et al., 2002); up to 182 ng l^{-1} in Florida (Owen et al., 2002; Gardinali et al., 2002, 2004); up to 42 ng l^{-1} in Puerto Rico (Carberry et al., 2006) and up to 1300 ng l^{-1} in the US Virgin Islands (Carberry et al., 2006). The high concentrations reflect the popularity of the product and the persistence of the compound in sea water (which has been estimated to be between 100 and 200 days; Konstantinou and Albanis, 2004). Sea-Nine 211® has been detected in Spanish, Danish and Greek marinas, on occasion at very high concentrations (3300 ng l^{-1} in Catalonia; Martinez et al., 2000). Rapid degradation of the compound (biological degradation $t_{1/2} < 1$ day; Ranke, 2002) renders distribution highly dependent on proximity to treated vessels. Maximum concentrations of up to 600 ng l^{-1} of dichlofluanid have been reported in Spanish ports and marinas (Martinez et al., 2000). Lower concentrations (<284 ng l^{-1}) have been recorded in Greek coastal waters (Sakkas et al., 2002). The relatively high octanol–water partition coefficient of the compound (Log $K_{ow} = 3.7$) renders greater association with the sediment rather than the aqueous phase (Voulvoulis et al., 2002). Whilst comparatively rapid degradation ($t_{1/2} < 5$ days) has been reported (Konstantinou and Albanis, 2004), sedimentary concentrations can be sustained outside of peak boating periods (Voulvoulis et al., 2000; Albanis et al., 2002). Chlorothalonil has been reported at up to 63 ng l^{-1} in Greek ports and marinas (Sakkas et al., 2002). It has also been identified at higher concentrations in UK estuarine waters (up to 1380 ng l^{-1}; Voulvoulis et al., 2002), although this contamination was attributed to agricultural rather than antifouling sources.

From the present study it is clear that for the herbicides investigated, they do not pose a threat to the coral communities. The question must, however, arise as to which products are used on the vessels in Chagos. It is likely that copper based antifoulants are applied.

Screening of the extracts using the full scan GC–MS data revealed the presence of steroids. Most prominent were the sterols cholesterol (cholest-5-en-3β-ol) and β-sitosterol (24α-ethylcholest-5-en-3β-ol) (Fig. 3). Standards were purchased from Sigma–Aldrich (UK) to confirm their identity. These compounds have been previously reported in sediment samples from Chagos (Readman et al., 1999). Through diagnostic ratios, these authors attributed sources of organic inputs to planktonic and/or benthic algal sources with a small terrigenous component. In the present study, water concentrations of cholesterol and β-sitosterol were lower for the off-shore station, in accord with reduced planktonic production and terrigenous inputs at this location.

The only synthetic compounds identified in the sample extracts (that were not present in the reagent blanks) were bisphenol A and 2,4,6-tritertbutyl phenol. These compounds are listed as a hazardous substance and a chemical for priority action, respectively, by the OSPAR Commission. Both compounds were, however, found in the methodological (cartridge) and field (off-shore water sample) blanks indicating that they leach from the SPE cartridges and contaminate the samples. This contamination problem is highlighted when working in pristine marine areas such as the Chagos Archipelago, and needs to be addressed in the monitoring of these priority substances, as has been requested for 2,4,6-tritertbutyl phenol (OSPAR Commission, 2006).

In summary, coastal water samples from the Archipelago revealed negligible contamination with levels generally below the limit of detection (<1 ng l^{-1}). Only in two harbour samples was an antifoulant (Irgarol®1051) detected at concentrations above the limit of detection (8 and 2 ng l^{-1}). With respect to the antifouling booster biocides and herbicides analysed, the coastal waters of Diego Garcia in the Chagos Archipelago appear virtually pristine posing negligible chemical threat to the coral communities. These data could be considered appropriate as a global reference baseline.

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Polybrominated diphenyl ether compounds in ringed, bearded, spotted, and ribbon seals from the Alaskan Bering Sea

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Polybrominated diphenyl ether compounds (PBDEs) are chemicals widely used as flame retardant additives in carpets and upholstery, and in plastics used in electrical appliances, televisions, and computers. It is thought that PBDEs enter the food chain by being released slowly into the air through the life of the products that contain them (Strandberg et al., 2001). Little is known about the toxicology of PBDEs; however, PBDEs and their congeners are structurally similar to polychlorinated biphenyls (PCBs) and thyroid hormones, and lab studies indicate that PBDEs may disrupt thyroid function and neurodevelopment (Darnerud, 2003; Viberg et al., 2004).

Furthermore, their appearance in human breast milk in the United States (She et al., 2000), Sweden, and Germany (Norén and Meironyté, 2000); in blubber of beluga whales (*Delphinapterus leucas*) in Canada (Stern and Ikonomou, 2000); and in blubber of harbor seals (*Phoca vitulina*) near San Franciscso (She et al., 2000) suggests that some PBDEs bioaccumulate. Lower brominated PBDEs were reported to biomagnify from polar cod (*Arctogadus glacialis*) to ringed seals (*Phoca hispida*) and from polar cod to beluga whales (Wolkers et al., 2004). Although decabromodiphenylether (Deca-BDE), the BDE congener produced in the greatest quantity, is easily eliminated and does not bioaccumulate (Hooper and McDonald, 2000; Kierkegaard et al., 1999), Deca-BDE can be converted to the lower congeners by exposure to sunlight (Watanabe and Tatsukawa, 1987; Sellström et al., 1998). There is some evidence that fish can metabolize Deca-BDEs to Hexa and Nona-BDEs (Kierkegaard et al., 1999). Some of these congeners (e.g., Tri-BDE to Hexa-BDE) appear to be highly bioaccumulative (Hooper and McDonald, 2000). There are also naturally occurring organohalogen compounds that contain bromine. These compounds are found in marine plants and invertebrates (Gribble, 1998, 1999). Little is known about these compounds but many may be more abundant than those from anthropogenic sources (Gribble, 1999).

Few data are available regarding levels of PBDEs in the United States. Many people living in Alaskan coastal communities eat seal tissues, including muscle and blubber. Seals are known to accumulate concentrations of persistent organochlorines (e.g., PCBs, DDTs) and may be bioaccumulating PBDEs as well. Levels of polybrominated diphenyl ethers (PBDEs) have not previously been reported in seals in Alaska. The objective of this analysis was to quantify levels of PBDEs in blubber from four species of seals (ringed, bearded, *Erignathus barbatus*; spotted, *Phoca largha*; ribbon, *P. fasciata*) that are consumed by humans and polar bears in Alaska.

Samples were collected from the subsistence seal harvest in 2003 at Little Diomede (65.7°N, 169.2°W) and Hooper

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